PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference JH/ml 032353wo	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/11027	International filing date *(day/month/year)* 06.10.2003	Priority date *(day/month/year)* 04.10.2002
International Patent Classification (IPC) or both national classification and IPC C12N15/09		
Applicant PROBIOGEN AG et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04.05.2004	02.12.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Aslund, J Telephone No. +31 70 340-4393

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/11027

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-34 as originally filed

Claims, Numbers

1-17 received on 13.10.2004 with letter of 12.10.2004

Drawings, Sheets

1/18-18/18 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1-10 (lack of industrial applicability)

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-16
	No:	Claims	17
Inventive step (IS)	Yes:	Claims	1-16
	No:	Claims	17
Industrial applicability (IA)	Yes:	Claims	11-17
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 1-10 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT, since said claims are not restricted to in vitro methods and do cover gene therapy (a method of treatment). Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

D1: ESPERET CORINNE ET AL: "Non-erythroid genes inserted on either side of human HS-40 impair the activation of its natural alpha-globin gene targets without being themselves preferentially activated." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 275, no. 33, 18 August 2000 (2000-08-18), pages 25831-25839, XP002225659 ISSN: 0021-9258

D2: KARREMAN S ET AL: "ON THE USE OF DOUBLE FLP RECOGNITION TARGETS (FRTS) IN THE LTR OF RETROVIRUSES FOR THE CONSTRUCTION OF HIGH PRODUCER CELL LINES" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 24, no. 9, 1 May 1996 (1996-05-01), pages 1616-1624, XP000616161 ISSN: 0305-1048

D3: WO 02/08409 A (OW DAVID W ;US OF AMERICA AS REPRESENTED B (US)) 31 January 2002 (2002-01-31)

D4: FENG Y-Q ET AL: "SITE-SPECIFIC CHROMOSOMAL INTEGRATION IN MAMMALIAN CELLS: HIGHLY EFFICIENT CRE RECOMBINASE-MEDIATED CASSETTE EXCHANGE" JOURNAL OF MOLECULAR BIOLOGY, LONDON, GB, vol. 292, no. 4, 1999, pages 779-785, XP000929622 ISSN: 0022-2836

D5: FUSSENEGGER M ET AL: "Genetic optimization of recombinant glycoprotein production by mammalian cells" TRENDS IN BIOTECHNOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 17, no. 1, January 1999 (1999-01), pages 35-42, XP004155532 ISSN: 0167-7799

D6: TRINH K R ET AL: "Site-specific and directional gene replacement mediated by Cre recombinase" JOURNAL OF IMMUNOLOGICAL METHODS,

ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL, vol. 244, no. 1-2, 20 October 2000 (2000-10-20), pages 185-193, XP004218458 ISSN: 0022-1759

D7: Sun et al (1994) J. Immunol. 152, 695-704

D8: Lang and Mocikat (1994) Mol. Gen. Genet. 242, 528-538

D9: Mocikat (1999) J. Immunol. Methods 225, 185-189

D10: WO9630498 (Xenotech Inc) 3rd of October 1996 (1996-10-03)

The following documents (D) were not cited in the international search report. Copies of the documents are annexed to the communication and the numbering will be adhered to in the rest of the procedure:

D11: Fukuta et al (2000) Archives of Biochemistry and Biophysics 378, 142-150

D12: Karpas et al (2001) Proc. Natl. Acad. Sci. 98, 1799-1804

Novelty - Article 33(2) PCT

The present application does not meet the requirements of of Article 33(2) PCT, because the subject-matter of claim 17 is not new.

Claim 17 relates to a target gene product obtainable by cultivating a cell as defined in claim 14 or 15. In the present case, such target gene product relates to a protein with human glycosylation pattern. However, since the product can not be discriminated from ANY human glycoprotein, the claim lacks novelty.

Amended claims 1-16 all relate to processes for genetically modifying an immortalized human cell or hybrid cell of B lymphocyte origin and the resulting cells as well as their use in protein production. Cited prior art documents D1-D10 disclose methods similar to the present application but do not relate to immortalized human cell or hybrid cell of B lymphocyte origin. Hence, novelty is acknowledged for claims 1-16.

Inventive step - Article 33(3) PCT

As pointed out in D11 page 143, there are important differences in glycosylation patterns between human cells lines and cells of murine origine. D11 further discloses differences in glycosylation pattern dependent on the method of immortalization of human cells. Hence, a technical problem with regard to expression of glycoproteins with human-like glycosylation pattern has been recognized in the prior art.

D12 discloses a human myeloma cell line which is suitable for generation of

hybridomas that stably express immunoglobulins at high yields. D12 states that the myelomas available in the prior art are not suitable for such purposes.
The present application makes use of cell lines with features similar to those in D12 and shows that highly expressed genes can be changed to a target gene of interested by homologous recombination using techniques described e.g. in D4. However, there are no clear incentives in the prior art to combine the teachings of D4 with D12.
Furthermore, it could not be predicted that the techniques of D4 would be readily applicable to cells such as those provided in D12. Consequently, an inventive step is acknowledged for claims 1-16.

CLAIMS:

1. A process for preparing a cell capable of stable high yield expression of a target gene product having an essentially human glycosylation pattern, which method comprises:
(a) selecting a human cell or human hybrid cell (starting cell) capable of stable high yield expression of a starting gene product being non-essential to the starting cell;
(b) screening for the locus of the starting gene product within the genome of the starting cell;
(c1) replacing the gene coding for the starting gene product with a first functional DNA sequence containing one or more recombinase recognition sites (RRS) to obtain a functionalized precursor cell; and
(d) integrating a second functional DNA sequence containing a DNA sequence coding for the target gene product into the functionalized precursor cell obtained in step (c1) by use of a recombinase recognizing the RRSs incorporated with the first functional sequence, or
(c2) directly replacing the gene coding for the starting gene product with a functional DNA sequence containing a DNA sequence coding for the target gene product.

2. The method of claim 1, wherein
(i) the starting cell secretes the starting gene product, preferably in an amount of at least 0.3 fmol/cell/d of a polypeptide chain and more preferably in an amount of more than 1 fmol/cell/d; and/or
(ii) the starting cell is a primary, immortalized or fusionated cell or a genetic modification thereof; and/or
(iii) if the starting cell is a human hybrid cell, the starting gene product is a human gene; and/or
(iv) the starting gene product is selected from secreted proteins such as antibodies, cytokines, hormones, enzymes, transport proteins, etc.

3. The method of claim 2 wherein the starting cell is an immortalized cell derived from B lymphocytes, preferably is a human myeloma or hybridoma, or human

hetero-hybridoma cell and most preferably is human-mouse hetero-hybridoma H-CB-P1 (DSM ACC 2104).

4. The method of claim 3, wherein the integration of the functional DNA sequence(s) is effected at a rearranged Ig locus, preferably at a rearranged immunoglobulin H locus or λ locus of said starting cell.

5. The method according to any one of claims 1 to 4 wherein the locus of the starting gene product
(i) is known or is determined by a screening procedure comprising microarray expression analysis, 2D protein gel electrophoresis, quantitative PCR, RNAse protection, northern blot, ELISA, western blot and combinations thereof; and/or
(ii) is selected as to provide for an essentially human glycosylation pattern.

6. The method according to any one of claims 1 to 5, wherein the replacement of the starting gene is effected
(i) by an one step replacement strategy, wherein the starting cell is contacted with a vector construct containing the first functional sequence, said first functional sequence replacing the gene coding for the starting gene product; or
(ii) in a two- or multi-step strategy, wherein the gene coding for the starting gene product is deleted or inactivated and subsequently contacted with a vector construct containing the first functional sequence, said first functional sequence being incorporated at the site of the deleted/inactivated starting gene product.

7. The method of claim 1 or 6, wherein the first functional DNA sequence
(i) comprises one or more RRS(s) selected from loxP, frt, attL and attR sites of lambdoid phages, and recognition sites for resolvases or phage C31 integrase,preferably RRS(s) capable of unidirectional integration such as modified loxP sites, frt sites, etc.; and/or
(ii) further comprises a functional sequences selected from marker sequences, secretion proteins, promoters, enhancers, splice signals, polyadenylation signals and IRES elements; and/or
(iii) is flanked in the vector by sequences that are homologous to the target gene or adjacent sequences.

8. The method of any one of claims 1 to 7, wherein
(i) the integration of the second functional DNA sequence is effected by delivering a recombinases recognising the RRS(s) present in the first functional sequence together with, shortly before or after delivery of the second functional sequence;
(ii) the integrase is selected from Cre, Flp, ϕC31 integrase, resolvase, etc.;
(iii) the target gene product is selected from enzymes, hormones, cytokines, receptors, antibodies, antibody domains, fusion proteins comprising the gene product mentioned before, etc.
(iv) the second functional DNA sequence further comprises functional sequences selected from promoter sequences, marker sequences, splice donor and acceptor sequences, recombinase recognition sequences differing from RRS of the first functional sequence, etc.

9. The method of any one of claims 1 to 6, wherein the gene coding for the starting gene product is directly replaced with a functional DNA sequence containing a DNA sequence coding for the target gene product.

10. A method for preparing a functionalized cell comprising the steps (a) to (c1) as defined in claims 1 to 6.

11. A functionalized cell as defined in claim 10.

12. A cell capable of high yield expression of a target gene product obtainable by the method of claims 1 to 9.

13. The cell of claim 12, wherein the target gene product is an antibody, preferably the cell is PBG04 (DMS ACC2577).

14. The cell of claims 11, 12 or 13, which is derived from H-CB-P1 (DSM ACC2104).

15. The cell of claim 13 or 14 further having its light chain inactivated or replaced with a gene coding for the same or a different target gene product.

16. A method for high yield expression of a target gene product which comprises cultivating a cell as defined in any one of claims 12 to 15.

17. A target gene product obtainable by cultivating a cell as defined in claim 14 or 15.